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Exploration of millet models for developing nutrient rich graminaceous crops

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Highlights

- Millets are nutritionally superior to other graminaceous crops.
- Use of ‘omic’ techniques to decipher nutritional traits of millets is discussed.
- Approaches to introgress these traits in other major cereals are shown.

Abstract

Protein-energy malnutrition and micronutrient deficiencies contribute to high mortality among considerable proportion of the current 7.2 billion global populations, especially children. Although poverty and diets poor in nutrition are prime reasons for prevalence of malnutrition, nutritionally dense crops offer an inexpensive and sustainable solution to the problem of malnutrition. Remarkably, millets are nutritionally superior to major non-millet cereals. They especially are rich in dietary fibers, antioxidants, phytochemicals and polyphenols, which contribute broad-spectrum positive impacts to human health. However, millets have received lesser research attention universally, and considering this, the present review was planned to summarize the reports available on nutrition profile of millets and non-millet cereals to provide a comparative insight on importance of millets. It also emphasizes the need for research on deciphering nutritional traits present in millets and to develop strategies for introgressing these traits into other conventional staple crops using germplasm and 'omics' technologies. In some millet species, excellent 'omics' and germplasm panels have started to get available which can act as a starting point for understanding as well as of introgressing healthful traits across millets and non-millet cereals.

Keywords: Millets; Nutrition; Gramineae; Food security; Genomics

Article outline

1. Introduction

Malnutrition is detrimental to human development, and irrespective of several preventive measures to ensure proper nutrition and diet, the human race, especially children are suffering from severe malnourishment. Stunting and underweight are the two prominent outcomes of malnutrition. Noteworthy, globally 161 million and 99 million children under the age of five are estimated to be stunted and underweight, respectively [1]. The data also indicates that about half of these stunted and two-third of underweight children live in Asia, whereas one third of stunted and underweight survive in Africa (Supplementary figure 1) [1]. According to Black et al. [2], 50% mortality among children under five is due to malnutrition and a recent study on stuntedness shows that child malnutrition rate in India is the highest in the world [3,4]. The poor nutritional content of the major non-millet cereals consumed is one of the major contributors to higher child malnutrition in India [5]. However, the case of undernourishment pertains not only in India but also in other countries that consumes major non-millet cereals (like rice and wheat) as their staple food. In addition to being nutritionally poor, these non-millet cereals have higher glycemic index (GI) which rapidly increases blood glucose levels resulting in hyperglycemia upon consumption. WHO has predicted that 347 million of global population contract diabetes and of the two major forms, type 2 diabetes accounts for around 90% of all diabetes worldwide [6]. The recent reports, revealing the occurrence of type 2 diabetes in children and projection of WHO that the death due to diabetes would increase one-fold by 2030, demand an immediate requirement of nutritional as well as low GI foods. Though preventive measures have been taken to circumvent malnutrition and diabetes by providing supplementary foods, promoting immunization, and monitoring feeding and caring practices, equal importance should be given to introduce nutritious and low GI foods as well as to improve the nutrient content of existing staple crops. Thus the need for alternative food crops which adequately caters the nutritional requirements and lower GI to prevent diabetes emphasizes the necessity of millets.

'Millets' is the generic term denoting an extraneous group of forage grasses known for their small-sized grains. Millets are mostly cultivated in the semi-arid areas of Asia and Africa, and being C_4 photosynthetic crops, millets are known to be climate-change resilient crops as they could grow efficiently in minimal conditions of moisture, high temperature and soils with poor nutrients [7,8]. In addition, millets possess several salient features such as high nutritive

properties, minimum vulnerability to pathogens and tolerance to drought and salinity [7]. Being the crops of shorter life-cycle, millets therefore are ideal staple crop for growing population. Millets are the members of grass family Gramineae, and are considered to comprise ten genera and at least fourteen species [9]. Of these, most important millets such as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*), and foxtail millet (*Setaria italica*) belong to Eragrostideae tribe, whereas other minor millets namely fonio (*Digitaria exilis*), kodo millet (*Paspalum scrobiculatum*) and tef (*Eragrostis tef*) belong Paniceae tribe. In addition to the differences in tribe, genus and species, these millets differ in genome size, ploidy and chromosome number (Supplementary table 1). Among the studied millets, foxtail millet has the smallest genome (~405.7 Mb; 2n=2x=18), whereas finger millet, 2509 Mb and pearl millet (~2450 Mb; 2n=2x=14) have the largest genomes [10,11]. The grain size and mass are also variable among the millets. Grains of pearl millet are relatively larger with an average thousand-seed weight ranging from 8 to 12 among germplasm accessions followed by proso millet (average thousand-seed weight ranging from 3 to 10 g), while foxtail millet has relatively small grains with an average thousand-seed weight ranging from 1.9 to 3.6 g [11].

Interestingly, genomes of these millets share a considerable synteny with non-millet cereals and bioenergy grasses since all these crops have evolved from a common Poaceae ancestor around 70 million years ago. Among these millets, foxtail millet and its wild relative green foxtail (*S. viridis*) were considered to be the model crops for studying the genetics and genomics of other millets, non-millet cereals and biofuel crops [12]. Considering this, the genomes of both the crops have been sequenced [13,14] and the availability of draft genome sequences has facilitated the large-scale development of genetic and genomic resources and demonstration of their utility in crop improvement [15].

Except foxtail millet and pearl millet, no other millets have been extensively studied and of note, the nutritional traits in these millets have not been systematically investigated till date. In this context, the present review summarizes the reports available on nutrition profile of millets and non-millet cereals to provide a comparative insight on the importance of millets. Further, the article also emphasizes the need for research on deciphering the nutritional traits of millets and to

develop strategies for introgressing these traits in to conventional staple crops. In millets, which are genetically closest to non-millet cereals, the feasibility of transferring these nutritional traits through integrated 'omic' approaches is higher and the resultant nutritionally enhanced graminaceous crops would cater the nutritional requirements of humans.

2. Nutrient profile of millets

Millets in general offer highly nutritious, non-glutinous and non-acid forming diets, although foxtail millet and proso millet have both glutenous and non-glutenous type of grains [16]. They possess high levels of proteins, minerals, vitamins and antioxidants, which imparts nutritional superiority of millets over non-millet cereals, and for such reasons millets are called 'nutritious millets' or 'nutricereals' (Table 1).

In addition to their high micro- and macro-nutrient contents, millets also encompass enhanced levels of low GI non-starchy polysaccharides and dietary fibers. Among different starches, high amount of slowly digestible starch and resistant starch accentuates millets as ideal diet for type 2 diabetics [20]. The forthcoming sections describe the composition of important nutrients and minerals in millets.

2.1 Proteins

Proteins are polymers of amino acids linked together by peptide bonds. As a nutrient, proteins serve as the source of amino acids, especially the essential amino acids which the human body cannot synthesize. Grains of millets and non-millet cereals contain large amounts of storage proteins which are utilized after germination as a source of nitrogen during the initial stages of embryo development. These storage proteins are fit for human consumption and so, the levels of seed storage proteins (SSP) are nutritionally important. SSPs are broadly classified into four categories according to their solubility characteristics, namely albumin (water soluble), globulin (soluble in dilute salt solution), prolamin (soluble in alcohol) and glutelin (extractable in dilute alkali or acid solutions) [21]. Albumin and globulin constitute major SSPs in dicots, whereas higher amount of prolamin and glutelin are found in monocots [22]. In millets, about half of the total grain proteins is prolamin and these are of four types, namely alpha-, beta-, gamma- and

delta-prolamins. Alpha-prolamins appear to be the major components in all millet species except fonio (*D. exilis*).

Comparison of proteins among millets and non-millet cereals showed that proso millet has highest levels of proteins (12.5g/100g), followed by foxtail millet (12.3g/100g) (Table 1). As of other non-millet cereals, wheat has a comparable level of protein (11.8g/100g) but rice comprised a much lower level of about 6.8g/100g. Further, wheat proteins are deficient in essential amino acids while almost all the millet proteins are reported to possess essential amino acids which are necessary for the prevention of protein-energy malnutrition (Table 2). A recent study on grain nutrient content in Indian cultivars of barnyard millet, little millet, finger millet, kodo millet and foxtail millet revealed that foxtail millet (cultivar 'RAU-8') had maximum protein (13.1%) in the seeds than other millets [24]. The analysis also showed that the levels of lysine (2.42 g/16 g N) and tryptophan (0.31 g/16 g N) were also high in foxtail millet cv. 'RAU-8' [24]. This report accords with the earlier findings of Mohamed et al. [25], in which foxtail millet was observed to contain higher amount of lysine.

2.2 Starch

In the seed endosperm of crop plants, starch is accumulated as the principal carbon and energy source for development, which also serves as the primary carbohydrate component in human diets [26]. Starch is comprised of two fractions, amylose and amylopectin, which gets hydrolyzed to simple sugars upon consumption. Based on the digestibility, starch is classified into three types namely, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) [27]. Major non-millet cereals are rich in RDS, and therefore, glucose is released immediately upon digestion resulting in increase in blood glucose levels. In case of millets, they are rich in SDS and RS, which resist digestion and get catabolized by the gut microbiota resulting in slow and steady release of glucose in the blood stream, thus reducing postprandial glycemic and insulinemic responses, reducing plasma cholesterol and triglyceride levels, improving whole body insulin sensitivity, increasing satiety and reducing fat storage [28]. Therefore, SDS and RS are important dietary components necessary for prevention of diseases related to dyslipidemia, type 2 diabetes, obesity and coronary heart disease.

Kumari and Thayumanavan [29] performed a comparative study of resistant starch isolated from minor millets on intestinal responses, blood glucose, serum cholesterol and triglycerides in rats. The study demonstrated that animals fed with a diet of native and treated starch from barnyard millet had the lowest blood glucose, serum cholesterol, and triglycerides compared to animals fed with non-millet cereals. In a similar study, dehulled and heat-treated barnyard millet was found to be advantageous for type 2 diabetics due to its low GI [30]. Bangoura et al. [31] evaluated physicochemical properties and content of RS in defatted white and yellow foxtail millet. The investigation showed that while foxtail millet comprised of 13.35% RS, whereas yellow foxtail millet contained 14.56% RS [32]. These studies demonstrate the implications for supplementing millets in diet to normalize glucose pressure in diabetics.

2.3 Lipids

Lipids contribute to the diet as a source of fat and essential fatty acids. Millets are an excellent source of three essential fatty acids, namely linoleic acid, arachidonic acid, and linolenic acid. The germ and aleurone layers are the main contributors to the lipid fraction in seeds, and lipid bodies were also found to line in cell periphery [33]. These lipid bodies enclose a matrix of storage triacylglycerols wrapped in a half unit membrane consisting of a single phospholipid layer and a few major proteins [34]. The germ layer itself provides about 80 percent of the total lipid [35]. Among millets, pearl millet has the maximum lipid content (3-6%), and finger millet, foxtail millet and kodo millet appeared to contain less fat. Moreover, about 75% of the fatty acids in pearl millet are unsaturated and linoleic acid proportion is particularly high (46.3%) [36]. Analysis of the composition of free and bound lipids in proso millet by Lorenz and Hwang [37] found that free lipids in flours ranged from 3.2 – 4.0% and in bran ranged from 3.4 – 6.8%. Linoleic acid, oleic acid and palmitic acid were the predominantly found free lipids, which contained hydrocarbons, sterol esters, triacylglycerols, diacylglycerols, and free fatty acids. In bound lipids, monogalactosyl diacylglycerols, digalactosyl diacylglycerols, phosphatidylethanolamine, phosphatidyl serine, and phosphatidyl choline have been identified [38].

In another study, lipid content and fatty acid composition of glutinous and non-glutinous varieties of foxtail millet were analyzed and found that linoleic acid comprised of about 70%

total fatty acids. Further, the main distinction between these varieties was observed to be due to the stearic and arachidic acid composition [38]. Similarly, linoleic (45.7%), oleic (24.7%), palmitic (16.7%) and stearic acids (8.2%) were identified in foxtail millet bran oil [39]. In addition, millets are rich in esterified steryl glycosides, monogalactosyl diglycerides, digalactosyl diglycerides, phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine [40].

2.4 Micronutrients

Millets are rich in vitamins and trace elements required for normal physiological functions of human body. Among vitamins, foxtail millet is rich in thiamin (0.59mg/100g), whereas maximum of riboflavin is found in proso millet (0.28mg/100g). The riboflavin content of rice and wheat were 0.04mg and 0.1mg, respectively (per 100g edible portion) (Table 1) which was far less than other millets especially pearl millet, foxtail millet and little millet. Table 3 summarizes the trace element level in millets. Phosphorus is a vital structural component of cell membranes and nucleic acids. In addition, phosphorus and calcium are involved in essential molecular processes such as bone mineralization, energy production, cell signaling and regulation of acid-base homeostasis. Almost all millets comprised excellent phosphorous levels, with maximum in foxtail millet (422mg/100g). Of note, finger millet is known for its abundance in calcium (398mg/100g). Magnesium serves as a co-factor for several enzymes and it is an active participant of enzymatic reactions, and kodo millet was observed to possess higher levels of magnesium (166mg/100g). Similarly, copper is rich in proso millet (5.8mg/100g), manganese in finger millet (5.49mg/100g) and chromium in little millet (0.240mg/100g). Iron and zinc contents in barnyard millet, little millet, finger millet, kodo millet and foxtail millet were extensively analyzed by Chandel et al. [24]. The iron content ranged from 4.4mg/100g in kodo millet to 4.6mg/100g in barnyard millet. Barnyard millet possesses higher levels of iron (4.0mg/100g) followed by finger millet (3.4mg/100g), little millet (3.2mg/100g), kodo millet (3.2mg/100g) and foxtail millet (2.7mg/100g) [24]. Zinc content was maximum in foxtail millet (4mg/100g), followed by barnyard millet (3.6mg/100g), little millet (3mg/100g) and finger millet (2mg/100g). In a similar study by Irén [34], zinc content in millets was analyzed and it was observed that foxtail millet comprises higher levels of zinc (2.1mg/100g). These reports indicate

that the micronutrient contents of millets are higher than the average micronutrient composition in major non-millet cereals.

3. Genetic and genomic resources of millets

Genetic resources are important for selection and breeding of elite cultivars rich in nutrition and other agronomic traits, and these resources will also be useful in identifying the genetic determinants of nutritional traits. The recent advances in genetic and genomic technologies along with other 'omics' approaches would promisingly expedite the discovery and transfer of genes/QTLs responsible for better nutrition profile from diverse genetic resources of millets. The Rockefeller Foundation of United States was the first to collect the millet germplasms for the use of breeders and researchers. However, at present the Consultative Group on International Agricultural Research (CGIAR) and Indian Council of Agricultural Research (ICAR) have the large *ex-situ* collection of millet germplasm. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India has the largest collection of 5949 finger millet accessions from 24 countries, 1535 foxtail millet accessions representing 26 countries, 842 proso millet accessions from 30 countries, 743 barnyard millet accessions representing 9 countries and 466 little millet accessions from 5 countries. In addition, ICRISAT also has a mini-core collection of 238 pearl millet accessions representing diversity of the global collection and all these accessions are available for crop improvement programs. Of note, the genome of pearl millet has also been sequenced by ICRISAT and the high-quality draft genome is expected to be released for the use of researchers (personal communication from Dr. Rajeev Varshney, ICRISAT, India).

India, being the largest producer of millets has several germplasm collection centers for Indian millet accessions. Among those, the National Bureau of Plant Genetic Resources (NBPGR) and All India Coordinated Millet Improvement Project (AICMIP) centres located in 14 regions of India were involved in conservation, evaluation and documentation of millet genetic resources. Recently, AICMIP has been renamed as All India Coordinated Pearl Millet Improvement Project (AICPMIP) with the mandate to preserve Indian pearl millet germplasm and produce improved varieties. In addition to these, National Institute of Plant Genome Research, India has a core collection of foxtail millet accessions collected from different eco-geographical regions of the world in addition to few wild cultivars of *Setaria*. Internationally,

millet germplasms were maintained at Institute of Crop Germplasm Resources of Chinese Academy of Agricultural Sciences, China, Plant Genetic Resources Conservation Unit of the United States Department of Agriculture, N.I.Vavilov Research Institute of Plant Industry, Russia, French agricultural research and international cooperation organization, France, National Institute of Agrobiological Sciences, Japan, National Genebank, Kenya Agricultural Research Institute, Kenya and Institute of Biodiversity Conservation and Research, Ethiopia.

In addition to the existing germplasm collections, advanced breeding lines and mutant genetic resources for few millet species are available. Krishnappa et al. [39] and Parashuram et al. [40] have demonstrated the genetic analysis of general combining ability (GCA) and heterosis by crossing popular finger millet lines and evaluated their breeding potential for establishment of superior recombinant inbred lines (RILs) for yield related attributes. Mauro-Herrera et al. [41] have derived a population of RILs (182 F_7 lines) from a cross between *S. italica* (foxtail millet) and *S. viridis* (green millet) and identified the genetic control of flowering in foxtail millet using a combination of SNP and SSR/STS markers. Cytoplasmic male sterility (CMS) lines of foxtail millet have been developed through inter-specific hybridization between *S. viridis* (accession N10; maternal parent) and *S. italica* (landrace *Daqingjie*; paternal parent). Three hybrids with a potential to utilize as CMS systems have been produced, though it has not been exploited in millet breeding [42]. Similarly, a genetic male sterile finger millet line INFM 95001 was developed using ethyl methane sulfonate (EMS)-induced mutagenesis in IE 3318 genotype background. The male sterile allele *ms1* of INFM 95001 was suggested to be useful in crossing self-pollinated finger millet and easier identification of hybrid progenies with heterotic pattern and facilitate recurrent selection [43].

Natural variations in waxy loci (*Wx*) were extensively studied in foxtail millet [44-46] and it was demonstrated that transposable element insertion-based mutation of *GBSS1* gene encoding granule-bound starch synthase 1, which serves as a key enzyme in amylase synthesis in endosperms resulted in decreased amylase content compared to the normal endosperm (>10%) [44]. The resultant ‘waxy’ phenotype is used in the preparation of sticky foods [46]. Similarly, *in vitro* culturing and regeneration of finger millet lines have resulted in the development of a popular somaclonal mutant ‘*SE7*’, which showed reduced plant height and increased grain yield

[47]. These mutants were extensively studied and cytokinin attenuation at the early inflorescence was identified to be the plausible reason for higher seed yield [48].

Advancements in DNA-based molecular markers have contributed to identification and tagging of various agronomically important genes and QTLs for construction of genetic maps, gene identification and plant breeding applications. Development of large-scale genome-wide markers, construction of maps and tagging of agronomically relevant genes and QTLs has been extensively performed in foxtail millet [15] and pearl millet [49], though little or no information is available in other millets. The availability of foxtail millet genome sequence has facilitated the development of ~20000 SSRs (simple sequence repeats) [50,51], ~500 EST-derived SSRs [52], ~5000 intron-length polymorphisms [53] and ~30000 transposable-elements based markers [54]. Noteworthy, the developed marker resources are available at Foxtail millet Marker Database (http://www.nipgr.res.in/foxtail.html) and Foxtail millet Transposable Elements-based Marker Database (http://59.163.192.83/ltrdb/index.html) [15]. High density physical maps were also constructed using these markers, which were useful in identification, tagging and map-based cloning of important genes/QTLs [15]. Recently, Jia et al. [55] have re-sequenced 916 diverse foxtail millet to identify 2.58 million SNPs and used 0.8 million common SNPs to construct a haplotype map of foxtail millet genome. This large data set of loci which are associated with a number of important agronomic traits in different environments and the loci that bear signatures of recent selection could serve as important source for further genetic improvement of both foxtail millet and other millets [55]. Similarly, RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) and SSR markers were used for the construction of genetic map in finger millet [56]. The map spans for both ‘A’ and ‘B’ genome of tetraploid finger millet and partially covers all the 18 chromosomes. However, lack of sufficient and informative marker densities and uniform distributions forms the major drawback in utilizing this genetic map for tagging important genes/QTLs in finger millet. Likewise, an concensus genetic map (418 cM) of pearl millet was constructed using 353 RFLP and 65 SSR markers mapped into seven linkage groups with ~85% of the markers occupying less than a third of the total map length [57]. Recently, Senthilvel et al. [58] identified ~300 polymorphic DArT™ (Diversity Array Technology) markers in three pearl millet RIL populations and have integrated this information with SSR data to construct individual genetic maps (with >300

marker loci, each). Similarly, few markers were developed in other millets, which are routinely used to assess population structure and genetic diversity [11], but no reports were available on the identification of genes/alleles/QTLs for nutritional and other agronomic traits in those millets.

4. Integrated 'omic' approaches for identification and introgression of nutrient traits in major non-millet cereals

A large genetic variation in nutrient composition exists among millets, but they have neither been extensively characterized nor exploited. Screening the available germplasm (core-collections) of all the millets for physio-chemical properties of individual nutrients and identification of high fidelity molecular markers followed by genome wide association study (GWAS) will lead to the identification of candidate genes/alleles/QTLs controlling nutritional traits. The existence of synteny and conservation among the grass family could be exploited to perform a comparative study among millets and other non-millet cereals for tracing the common genes involved in nutrition biosynthesis pathways. Once identified, these genes/alleles/QTLs present in the diverse germplasm can be incorporated into the elite cultivars through either transgene based or molecular marker assisted breeding approaches to enhance the nutrients in grains. The genetic close-relatedness of millets with major non-millet cereals such as rice, wheat, barley, sorghum and maize also facilitates the introgression of these nutritional traits from millets into the major non-millet cereals. The integrated role of genomics, transcriptomics, proteomics, metabolomics and ionomics is imperative towards achieving this goal (Figure 1).

4.1 Genomics

With the advent of next-generation sequencing (NGS), genomics and transcriptomics have advanced to the next level in high-throughput analysis of genes and transcripts, respectively. NGS has widely been used for *de novo* sequencing, whole genome sequencing (WGS), whole genome resequencing (WGRS), genotyping by sequencing (GBS), transcriptome and epigenetic analysis, and therefore, NGS would serve as a powerful tool for identifying the genetic basis of nutritional traits in millets (Figure 2). Foxtail millet is the first among millets to get its genome sequenced [13,14]. Foxtail millet cv. 'Zhang gu' was sequenced by Zhang et al. [13] using Illumina second generation sequencers with ~86% genome coverage. A total of 16,903 contigs

and 439 scaffolds were produced spanning a total length of 423 Mb with 28 Mb (6.6 %) gaps and a predicted genome size of ~485 Mb [13]. A photo-thermo-sensitive male sterile line 'A2' was also sequenced and compared with 'Zhang gu' to identify large-scale SNPs, insertion/deletion polymorphisms (InDels) and structural variations (SVs). A total of 38,801 genes were identified, out of which ~82 % were found to be expressed. Further, a genetic linkage map was constructed from a cross of 'Zhang gu' and 'A2', mapped with 751 markers including 118 SNPs and 641 SVs [13]. Similarly, Bennetzen et al. [14] sequenced foxtail millet accession 'Yugu1' and green foxtail accession 'A10' using ABI3730xl capillary sequencer. A total of 5,736,559 reads were generated and assembled to achieve ~80% genome coverage. The analysis identified 24,000–29,000 expressed genes and the genome size was computed as 396.7 Mb distributed in nine chromosomes. In addition to this, 4.2 Mb in 327 scaffolds (mostly <50 kb in size) was also identified. Further, the RILs from foxtail millet 'B100' X green foxtail 'A10' populations were sequenced and 3,149,093 SNPs were identified. A total of 992 SNPs were then scored for segregation in RIL population and a high-resolution genetic map was constructed [14].

The availability of draft genome sequence has facilitated genome-wide identification and characterization of several stress-responsive gene families in foxtail millet [59-65]. However, identification of genes responsible for nutrient content is in initial stage. Comparative genomics approach was used to identify the genes encoding for prolamins and starch biosynthesis in foxtail millet using rice genes (Unpublished data). Prolamins are a class of seed storage proteins present predominantly in monocot seeds and are major source of nitrogen and sulphur [66]. Sixteen prolamin-encoding genes were identified in foxtail millet (called '*setarins*') using computational approaches (Unpublished data). Sequence alignment of setarin proteins with prolamins of other millets and cereals showed that setarins share least homology with other crops, and phylogenetic analysis demonstrated the out-grouping of setarins due to less homology. *In silico* promoter analysis showed that all the *setarin* genes have conserved prolamin box in addition to few common cis-regulatory elements. Expression profiling of *setarin* genes in different tissues of foxtail millet using qRT-PCR indicated over-expression of *setarin* genes in developing seed and less or no expression in other tissues (Unpublished data). Similarly, key gene families involved in starch biosynthesis pathway namely adenosine 5' diphosphate-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE), starch debranching enzyme

(DBE), starch phosphorylase (PHO) and starch disproportionating enzyme (DPE) have been identified in foxtail millet using homologous rice genes (Unpublished data). A total of 6 *AGPase*, 12 *SS*, 4 *SBE*, 3 *DBE*, 2 *PHO* and 3 *DPE* encoding genes were identified and physically mapped onto foxtail millet chromosomes. Their gene structure, phylogeny and domain composition were analyzed using *in silico* approaches. Expression profiling of candidate genes at various time points of seed development in different cultivars provided insights to delineate their role in high RS content (Unpublished data). Overall, the studies on prolamin-encoding and starch biosynthesis genes provided preliminary clues towards understanding the molecular genetics and genomics of high protein and starch content in foxtail millet.

Recently, the genome of tef (*E. tef*) has also been sequenced [67], which is a gluten-free crop with high protein, vitamin and mineral content. In addition, the genome of pearl millet has also been sequenced (personal communication from Dr. Rajeev Varshney, ICRISAT, India).The genome of tef accession 'DZ-Cr-37' sequenced using Ilumina HiSeq2000 and 454-FLX platforms generated a total of 40 Gbp from single- and paired-end reads resulting in 44-fold coverage (~80% genome coverage). The size of assembled tef genome was found to be 672 Mbp and in addition, a total of 49600 SSR markers were also developed in tef genome. Further, the tef genome was also evidenced to comprise of transposable elements (6%), retroelements (class I transposable elements; 3.9%) and DNA transposons (class II transposable elements; 2%). In addition, 80 rRNAs, 1184 tRNAs, 570 miRNAs and 834 snRNAs have also been identified in tef genome. However, no large-scale mining of molecular markers for identification of genes/QTLs or pilot-scale studies on genes and gene families responsible for nutrition traits have been conducted in tef except the identification of prolamin family genes [67]. Tef genome was found to encode for 23 prolamin genes, which were mostly tandem duplicates. These prolamins were classified into three groups according to their phylogenetic relationships with prolamins of other grasses.

Identification of major-effect genes and QTLs responsible for nutrition content and quality could also be identified using NGS. The draft genome sequence of foxtail millet has facilitated large-scale development of different molecular markers including microsatellites [50-52], intron-length polymorphisms [53], miRNA-based markers [68] and transposable elements-based

markers [54]. These genomic resources can be utilized in performing association mapping through GWAS for mapping the QTLs by using historic linkage disequilibrium to identify statistically significant phenotype-genotype associations [69]. Candidate genes can also be identified using high resolution genetic mapping in which sequencing-based mapping is performed in combination with bulked segregant analysis. This approach has been proved successful in identifying major gene responsible for blast resistance (*Pii*) and QTLs responsible for seedling vigour in rice [70,71]. After identifying the genes, it shall be introgressed into any desired crop(s) by utilizing markers for screening the progenies which carry the most favorable combination of alleles. Both genotyping arrays and NGS strategies have been proved efficient for introgressing target loci into elite varieties [72]. This could also be possible through transgene-based approaches [73,74]. Although this would be the final step in generating elite crop varieties with improved nutrient content, other 'omic' technologies play a crucial role prior to this.

4.1.1. Comparative genomics

Comparative genomics takes the advantage of genetic conservedness between closely related species for identification of genes and genomic regions using genomic collinearity and synteny maps. Synteny maps are constructed using common set of orthologous sequences, which help in translating the genomic information from well-studied species to less-researched species [75]. Several marker- and gene family- based synteny maps have been constructed between the genome of foxtail millet and genomes of rice, maize, sorghum and *Brachypodium* [15]. These sequence-based synteny maps have revealed higher genomic collinearity between foxtail millet and sorghum, followed by foxtail millet and maize, and foxtail millet and rice. An example demonstrating the use of comparative genomics approach is the identification of maize ortholog of *teosinte branched1* (*tb1*) gene in foxtail millet, which regulates vegetative branching or tillering [76]. Overall, comparative genomics strategies will be effective in identifying orthologous millet genes and QTLs in the genome of non-millet cereals for improvement of nutrition content.

4.2 Transcriptomics

NGS has revolutionized transcriptome sequencing than genome sequencing. Due to the benefit of cost effectiveness and high coverage, transcriptome sequencing of most of the crop plants have been performed to identify genes and gene families involved in various physiological, molecular and stress-responsive activities. In addition, RNA-sequencing (RNA-seq) reveals the relative abundance of transcripts in a given sample (tissue- and/or condition-specific) and also allows high-throughput development of molecular markers. Before the availability of RNA-seq platforms, trancriptomics was executed by comparative approach through cDNA-AFLP, suppression subtraction hybridization (SSH) and microarray analysis. In foxtail millet, cDNA-AFLP and SSH methodologies were demonstrated to be useful in identifying differentially expressed transcripts during salinity and dehydration stresses [77-79]. Whole transcriptome sequencing in millets was also first reported in foxtail millet, in which RNA-seq has been performed in root, leaf, stem and spica samples using Illumina GA II platform [13]. The average size of foxtail millet transcripts was predicted to be 2,522 bp with an average intron length of 442 bp, average exon length of 256 bp and average exons per gene as 4.3. The transcriptome data showed that ~81.7% of foxtail millet genes were expressed, whereas 1,367 were pseudogenes [13]. Similarly, the transcriptome of tef tissues have also been sequenced along with its genome using 454-FLX technology [67]. A normalized transcriptome library prepared from roots and shoots of tef seedlings produced 27756 gene clusters and 38333 transcripts, whereas non-normalized library obtained from various tef tissues subjected to drought and water-logging treatments produced 28113 gene clusters and 88078 transcripts. Comparative analysis of both transcriptomes revealed the presence of distinct, stress-related transcripts [67].

Among millets, finger millet transcriptome has been extensively studied. Rahman et al. [80] sequenced the RNA samples of salinity stress treated contrasting finger millet cultivars (CO 12 - susceptible; Trichy 1 - tolerant) using Ion PI™ Chip technology. A total of 12,025,195 (CO 12 - control); 3,786,153 (CO 12 - stress); 6,557,442 (Trichy 1 - control) and 5,544,738 (Trichy 1 - stress) reads were obtained [80] and were mapped onto rice (~50%) and foxtail millet (~18%) genomes. More than 80 % of unmapped reads with foxtail millet demonstrated the close genetic relatedness of finger millet with rice than foxtail millet. Further, the study has identified differentially expressed genes in response to salinity stress in two contrasting finger millet

cultivars, which includes transporters, transcription factors, genes involved in cell signaling, osmotic homeostasis and biosynthesis of compatible solutes [80]. Considering the higher levels of calcium accumulation in finger millet, Singh et al. [81] sequenced the total RNA in spike tissues of two finger millet genotypes differing in their grain calcium content (GP-1 – low calcium genotype; GP-45 – high calcium genotype) using Illumina HiSeq 2000 platform. Of the 120130 contigs in high calcium genotype, 82 were identified to be calcium sensor genes and through bioinformatic analyses, these genes were classified into 8 gene families, namely CaM and CaMLs, CBLs, CIPKs, CRKs, PEPRKs, CDPKs, CaMKs, and CCaMK. Comparing the relative abundance of transcripts revealed that 24 calcium sensor genes were highly expressed in high calcium genotype [81,82]. These studies exemplify the application of *de novo* transcriptome data assembly and analysis, and encourage transcriptome sequencing in all millets for comprehensive identification and functional characterization of genes and gene families responsible for nutrition content. Since grains of millets are ultimate source of nutrition, transcriptome at different levels of seed development should be analyzed to identify the candidate genes and to understand their roles in nutrient biosynthesis as well as accumulation.

4.3 Proteomics and metabolomics

In nutritional context, proteomics and metabolomics are the integral parts and key players among ‘omic’ disciplines, as they are imperative for characterizing the biomolecules and trace elements which are considered nutritive. Although no attempt has been made to understand the concept of nutrition and analyze the nutrients on proteomics and metabolomics perspective, these studies will certainly enable the determination of proteins and metabolites in millet seeds towards identifying their changes in response to several factors. An important application is that, the proteomics and metabolomics shall be utilized in nutrient as well as metabolite profiling of all the available millet genotypes and digital labelling thereof for easy identification of versatile seeds. Further, these tools will assist in the development of biomarkers for nutritional traits. Since the physiological and metabolic pathways in crop plants are highly intricate and interrelated, proteomic and metabolomic approaches would assist in identifying the unique genes with nutritional properties through bottom-up approach. In addition to study the composition and characteristics of dietary nutrients, proteomic and metabolomic tools can also be used to analyze

the postprandial effects such as, digestion and absorption of nutrients in the gastrointestinal tract, nutrient metabolism and its regulation, and functions of nutrients in growth and health.

4.4 Ionomics

Ionomics denotes the determination of elemental composition of an organism and change in their composition in relation to physiological, developmental, environmental, and genetic factors [83]. The accumulation of nutrients is an intricate process regulated by a network of genes and gene products playing crucial roles in imbibing, binding, translocating and sequestering the nutrients. The activities of these genes are influenced by several elements and to understand the mechanism of regulation of these elements, ionomics is essential. In nutritional perspective, ionomics will serve as an efficient tool in understanding the mechanism of mineral transport in millets by identifying the candidate transporter genes and their further functional validation [82]. Though ionomic studies are in their preliminary stage, it has the potential to extrapolate the role of ionome in determining the nutrient levels. This knowledge will facilitate the development of plants for biofortification and also for developing necessary experimental work-plans to ensure effective bioavailability of nutrients.

5. Conclusions

Millets can aptly be termed versatile crops due to their nutritious properties, low GI and climate-change resilient characteristics. Since times, only vitamins, minerals, essential fatty acids and fibers were considered to be responsible for conferring health benefits, but recent studies have demonstrated that these elements could also act in combination with a number of other bioactive molecules to exert positive effects. These bioactive molecules include (i) resistant starch, oligosaccharides, and lipids; (ii) antioxidants such as phenolic acids, avenanthramides, and flavonoids, (iii) hormonally active compounds including lignans and phytosterols, and (iv) anti-nutrients such as phytic acid and tannins [83]. Interestingly, millets are extremely rich in all these nutrients as well as in bioactive molecules which qualify them to be in functional food category. By providing dietary fibers, proteins, energy, minerals, vitamins, and antioxidants essential for human health, millets also qualify to be called ‘nutraceuticals’. Evidences are there in support of millets playing prime role in preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol, and rate of

fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk [83-85]. Until recently millets have received very little scientific interest [86], but recently a number of new initiatives have been announced both at regional and global scale to harness the health benefiting potential of millets. All these research efforts will help deciphering the nutritional traits of millets and with the help of germplasm and other existing 'omics' technology will introgress them in major non-millet cereals. Although there is an initial understanding on nutrient biosynthetic pathways, a comprehensive experimental framework that integrates transcriptomics, genomics, proteomics, metabolomics, ionomics and bioinformatics is essential to efficiently investigate specialized nutrient pathways in millets.

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Figure Captions

Figure 1. Integrated circuit of different 'omics' approaches for improving the nutrition content of major cereals. An orchestrated role of all the omics technologies is required to extrapolate the nutritional traits of millets and introgress to cereals.

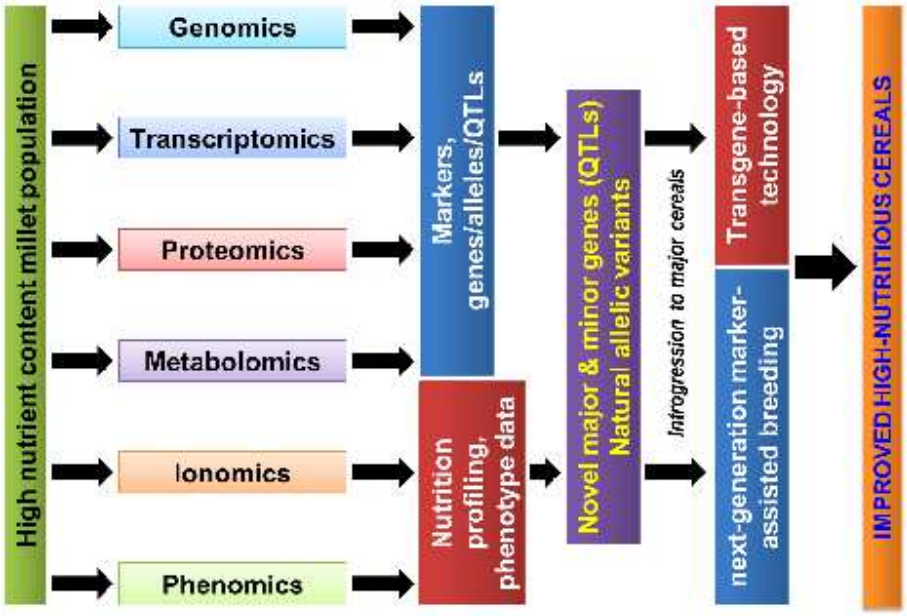
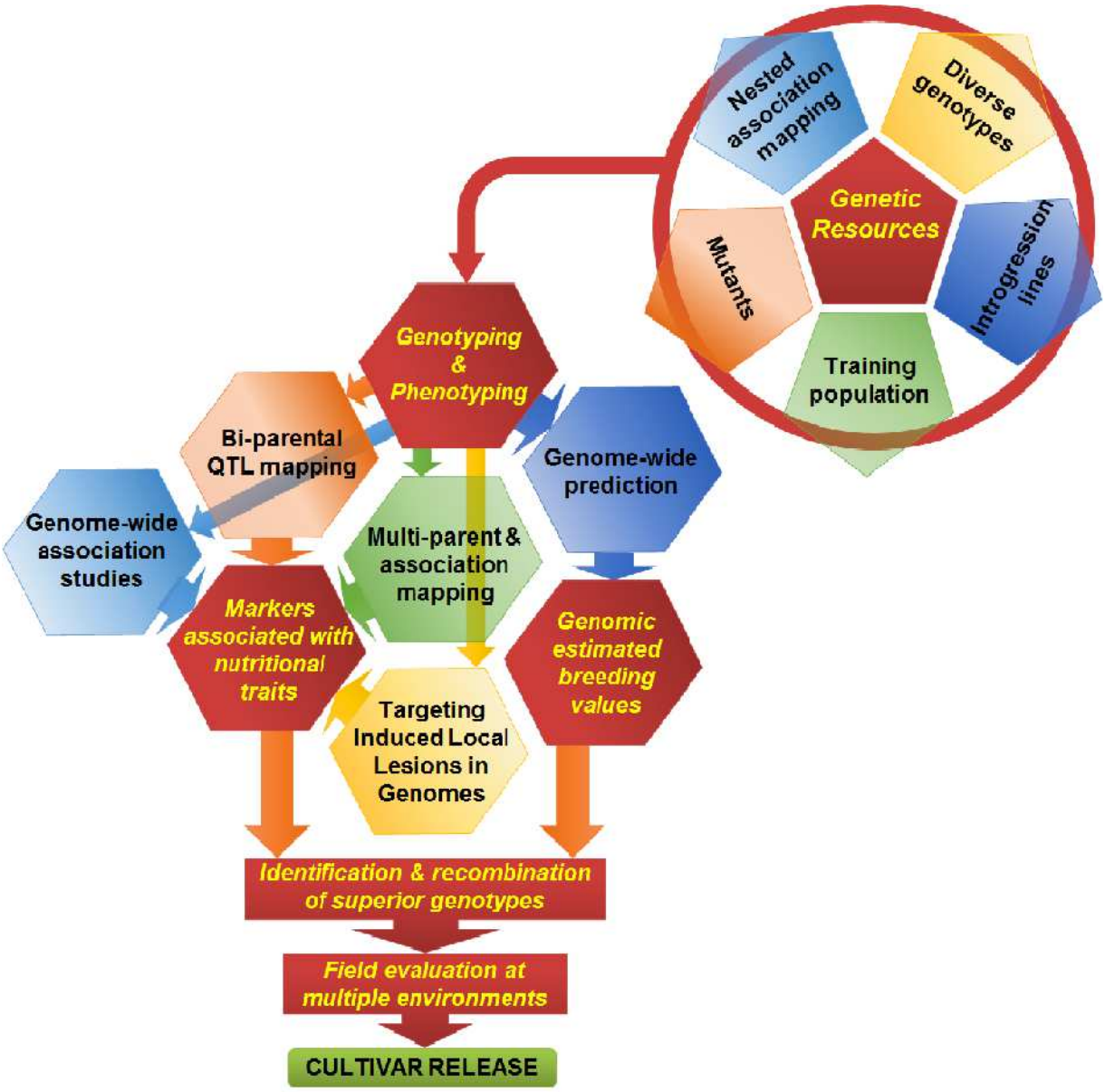


Figure 2. Flowchart demonstrating the role of NGS in expediting genomics-assisted breeding for improving nutrition content in major cereals.



Tables

Table 1 Proximate nutrient composition of millets and non-millet cereals.

Grain	**Composition (per 100g grain) [**17-19]							
	Protein (g)	**Fat (g)**	**Ash (g)**	**Crude fiber (g)**	**Carbohydrate (g)**	**Energy (kcal)**	**Thiamin (mg)**	**Riboflavin (mg)**
Pearl millet	11.8	**4.8**	2.2	2.3	67.0	363	0.38	0.21
Finger millet	7.3	1.3	2.7	3.6	72.0	336	0.38	0.21
Foxtail millet	**12.3**	**4.3**	**3.3**	8.0	60.9	351	0.42	0.19
Proso millet	**12.5**	3.1	1.9	7.2	70.4	**364**	**0.59**	0.11
Little millet	7.7	4.7	1.5	7.6	67.0	329	0.41	**0.28**
Barnyard millet	6.2	2.2	**4.4**	**9.8**	65.5	300	0.30	0.09
Kodo millet	8.3	1.4	2.6	**9.0**	65.9	353	0.33	0.10
Rice	6.8	0.5	0.6	0.2	**78.2**	362	0.41	0.04
Wheat	11.8	1.5	1.5	1.2	71.2	348	0.41	0.10
Sorghum	10.4	3.1	1.6	2.0	70.7	329	0.38	0.15

Table 2 Essential amino acid composition of millet proteins.

Grain	**Amino acid composition (mg/g) [**23]									
	Ile	**Leu**	**Lys**	**Met**	**Cys**	**Phe**	**Tyr**	**Thr**	**Trp**	**Val**
Pearl millet	256	598	214	154	148	301	203	122	122	345
Finger millet	275	594	181	194	163	325	-	191	191	413
Foxtail millet	475	1044	138	175	-	419	-	263	61	431
Proso millet	405	762	189	160	-	307	-	61	49	407
Little millet	416	679	114	142	-	297	-	49	35	379
Barnyard millet	288	725	106	133	175	362	150	35	63	388
Kodo millet	188	419	188	94	-	375	213	63	38	238

- Data not available.

Table 3 Trace element composition in millet grains.

Grain	Trace elements composition (mg/100g dry matter) [23]								
	P	**Mg**	**Ca**	**Fe**	**Zn**	**Cu**	**Mn**	**Mo**	**Cr**
Pearl millet	379	137	46	8.0	3.1	1.06	1.15	0.07	0.023
Finger millet	320	137	398	3.9	2.3	0.47	5.49	0.10	0.028
Foxtail millet	422	81	38	5.3	2.9	1.60	0.85	-	0.070
Proso millet	281	117	23	4.0	2.4	5.80	1.20	-	0.040
Little millet	251	133	12	13.9	3.5	1.60	1.03	-	0.240
Barnyard millet	340	82	21	9.2	2.6	1.30	1.33	-	0.140
Kodo millet	215	166	31	3.6	1.5	5.80	2.90	-	0.080

- Data not available.